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Exploring Cellular Morphology of *Thermoplasma acidophilum* by Cryo-Electron Tomography with Volta Phase Plate

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Thermoplasma acidophilum is a thermoacidophilic archaeon which inhabits harsh environment such as 60ºC and pH 2 [1]. It has been already reported that transmission electron microscopy (TEM) observation of the *Thermoplasma acidophilum* showed unique morphological property that the organism does not possess cell wall unlike typical archaeal cells. Therefore, it seems that *Thermoplasma acidophilum* is highly dynamic and shows irregular shape due to lacking cell wall. However, details of cellular morphology and intracellular features of *Thermoplasma acidophilum* are still unclear.

For understanding of cellular function, not only studies of morphology but also studies of molecular sociology of cells and macromolecular assemblies *in situ* are important. Cryo-electron tomography (CET) of vitrified, frozen-hydrated cells is an unique methods of studying the three dimensional structure of pleomorphic objects, such as organelles or cells preserved in their natural, cellular environment, with a resolution of low nm range [2]. However, low signal-to-noise ratio is a drawback of CET.

A Volta phase plate has been developed to improve image contrast in cryo-transmission electron microscopy (cryo-TEM) [3]. The Volta phase plate is effective not only single particle analysis [4] but also CET [5]. In the CET of frozen hydrated bacterial cells, the Volta phase plate showed better image contrast improvement than energy filter [6]. Additionally, the visibility improvement provided by Volta phase plate allows the identification and localization of macro protein complexes such as 26S proteasome in the intracellular environment [7]. In this study, we investigated cellular morphology of *Thermoplasma acidophilum* by CET with Volta phase plate.

As a specimen, cultivated *Thermoplasma acidophilum* cells were frozen with BSA coated 10 nm Au fiducial marker by plunging into liquid ethane/propane mixture using Vitrobot. TEM observation was carried out with Titan Krios equips with K2 direct detector, energy filter and Volta phase plate. The tilt-series images were recorded with a tilt-range ±60º and tilt increment 2º. The pixel size at specimen level was 3.42 Å. Tomograms were reconstructed by MATLAB using the AV3 and TOM-toolbox.

Tomographic slice images showed that intracellular space is well crowded with granular macro protein complexes which seemed to be 70S ribosome. Tomographic slice images also showed repetitive filamentous bundle and membrane features.

For the *in situ* structural studies of these membrane features, subtomogram averaging of these membrane features was carried out. The averaged membrane features were classified into two different shapes of protein complex such as ‘spike shape’ and ‘ball shape’.

Thus, by applying CET with Volta phase plate for frozen-hydrated *Thermoplasma acidophilum,* cellular architecture and macro molecular structure *in situ* could be visualized. As a future challenging for

further understanding of cellular functions, it seems to be important to identify or annotate the visualized structures in cells.

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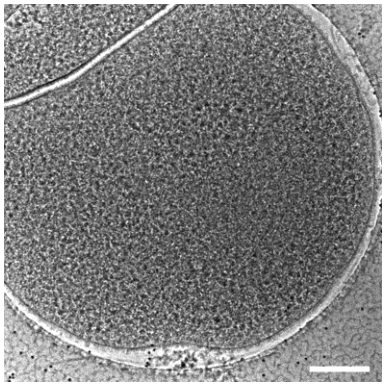

Figure 1. Cryo-electron micrograph of plunge-frozen *Thermoplasma acidophilum*. Scale bar: 200 nm.

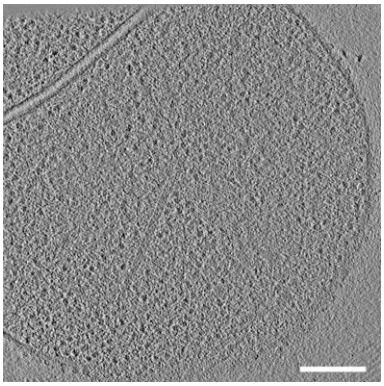

Figure 2. Tomographic slice image from plunge-frozen *Thermoplasma acidophilum*. Scale bar: 200 nm.

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